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DEVRIJ, W; POOLMAN, B; AZZI, A; KONINGS, WN

Published in:
Antonie van Leeuwenhoek: Journal of Microbiology

Publication date:
1985

Structural and functional aspects of cytochrome *c* oxidase from *Bacillus subtilis* W 23

W. DE VRIJ[1], B. POOLMAN[1], A. AZZI[2] and W. N. KONINGS[1]

[1] *Department of Microbiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands*

[2] *Medizinisch-Chemisches Institut, University of Bern, Bühlstraße 28, CH-3000 Bern 9, Switzerland*

Eukaryotes and many prokaryotes contain cytochrome *c* oxidase as the terminal component of the electron transport chain. Although bacterial cytochrome *c* oxidases are structurally less complex than eukaryotic oxidases, the functional properties of both types of oxidases show striking similarities. Recently, we have determined some characteristics of *Bacillus subtilis* cytochrome *c* oxidase purified to homogeneity by cytochrome *c* affinity chromatography (De Vrij et al., 1983) or a combination of anion-exchange chromatography and gel filtration (De Vrij et al., 1985). The purified enzyme showed absorption maxima at 414 nm and 598 nm in the oxidized and at 443 and 601 nm in the reduced form. Upon reaction with carbon monoxide of the reduced purified enzyme the absorption maxima shifted to 431 nm and 598 nm. The purified enzyme is composed of three subunits with apparent mol wts of 57, 37 and 21 kdal. The reaction catalysed by this oxidase was strongly inhibited by cyanide, azide and carbon monoxide, characteristic for an aa_3-type oxidase. Besides yeast cytochrome *c*, phenazine methosulphate and *N, N, N', N'*-tetramethyl-*p*-phenylene diamine (TMPD) are effective electron donors to this oxidase. Immunoabsorption experiments indicated a transmembranal localization of the protein in the cytoplasmic membrane. We investigated the kinetics of cytochrome *c* oxidase from *B. subtilis* using yeast cytochrome *c* as substrate at different ionic strengths. The results show that the maximum turnover number of the purified enzyme in the solubilized state is independent of the ionic strength. However, increase of the ionic strength results in an increase of the K_m for cytochrome *c*, indicating electrostatic interaction between enzyme and substrate.

The bacterial enzyme exhibits positive cooperativity at low ionic strength, which is lost at high ionic strength. This cooperativity cannot be related to the aggregation state of the enzyme, which independently of ionic strength, appears to be present in a dimeric form. Under more physiological conditions, i.e. in the cytoplasmic membrane the enzyme exhibited monophasic kinetics.

The energy-conserving properties of cytochrome *c* oxidase from *B. subtilis* were investigated in reconstitution experiments. *B. subtilis* cytochrome *c* oxidase incorporated in artificial membranes generates an electrical potential (inside negative) upon addition of an electron donor. The magnitude of the $\Delta\psi$, generated by cytochrome *c* oxidase, was calculated from the uptake of the lipophilic cation tetraphenyl phosphonium (TPP^+). Upon addition of ascorbate-cytochrome a $\Delta\psi$ of -50mV could be generated. Subsequent addition of the electron mediator TMPD leads to $\Delta\psi$-values of -80 to -90 mV, indicating that most probably TMPD accelerates the electron transition from ascorbate to cytochrome *c*. Addition of nigericin, which catalyses electroneutral exchange of protons for K^+, results in an increase of the $\Delta\psi$ to values of -100 mV. This indicates that besides an electrical potential also a transmembrane proton gradient is generated during the reaction. This was confirmed by flow-dialysis experiments using acetate to determine a ΔpH, inside alkaline. Using ascorbate-TMPD-cytochrome *c* as an electron donor system a ΔpH of 40 to 50 mV (inside alkaline) could be calculated, which slightly increased by the addition of valinomycin.

DE VRIJ, W., AZZI, A. and KONINGS, W. N. 1983. Structural and functional properties of cytochrome *c* oxidase from *Bacillus subtilis* W 23. — Eur. J. Biochem. **131**: 97–103.

DE VRIJ, W., POOLMAN, B., AZZI, A. and KONINGS, W. N. 1985. Cytochrome *c* oxidase of *Bacillus subtilis*. — Methods Enzymol. (in press).